PHYSIOLOGICAL ANALYSIS OF THE CORTICAL RESPONSE OF THE SEA URCHIN EGG TO STIMULATING REAGENTS. II. THE PROPAGATING OR NON-PROPAGATING NATURE OF THE CORTICAL CHANGES INDUCED BY VARIOUS REAGENTS

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In the previous paper of this series, it was concluded that an invisible change of a propagating nature must occur at the time of fertilization in sea urchin eggs in order that the cortical granules be broken down. This change was called the "fertilization-wave," as in the case of Oryzias eggs (Yamamoto, 1944). It was suggested that sodium choleinate and wasp-venom do not initiate the fertilization-wave, although they exert their influence upon the cortical granules to induce their breakdown. This means that the artificially induced breakdown of the granules does not always reveal itself as a consequence of the fertilization-wave. The question then arises as to the presence or absence of the fertilization-wave in the cortical changes induced by various other artificial stimuli. The possibility must be considered that some stimulating reagents provoke the fertilization-wave, which leads to the breakdown of the cortical granules, while others act directly to cause the breakdown of the cortical granules, without inducing a fertilization-wave.

An investigation has been made to determine whether the effects of various stimulating reagents are of a propagating nature. If they are of a propagating nature, it may very probably indicate that the fertilization-wave is provoked. On the contrary, if they are of a non-propagating nature, it may be clear that the fertilization-wave does not occur. It is the purpose of the present paper to report the results of these experiments.[1]

Material and Methods

The sea urchin, *Hemicentrotus* (*Strongylocentrotus*) *pulcherrimus* was used throughout. The eggs to be used for experiments were carefully collected in the usual manner and only such eggs as showed good fertilizability in control experiments were used. Wasp-venom, sodium choleinate, urea, glycerine, sucrose, distilled water, detergents and fatty acids were employed as stimulating reagents.

In order to investigate whether the effect of a stimulating reagent is of a propagating nature, the following method was adopted. The egg surface was partially exposed, as described below, to the stimulating reagent for an appropriate time and then washed with sea water. The unexposed surface was examined with the

[1] It may be added that elaborate works on conduction of the block to polyspermy have been done by Rothschild and Swann (*Exp. Cell Research*, **2**: 137, 1951; *J. Exp. Biol.*, **28**: 403–416, 1951; *J. Exp. Biol.*, **29**: 469–483, 1952).

oil-immersion objective. When the cortical granules in the exposed part were broken down and those in the unexposed part were intact, it was considered that the effect of the reagent was not of a propagating nature. But when the cortical granules in the whole surface, including the unexposed part, were broken down, the effect of the reagent was conceived to be of a propagating nature.

Partial exposure of the egg surface to the reagent was achieved by means of the technique diagrammed in Figure 1a. The jelly-coats of the eggs were first removed by treating the eggs with a weak solution of HCl in sea water. It was

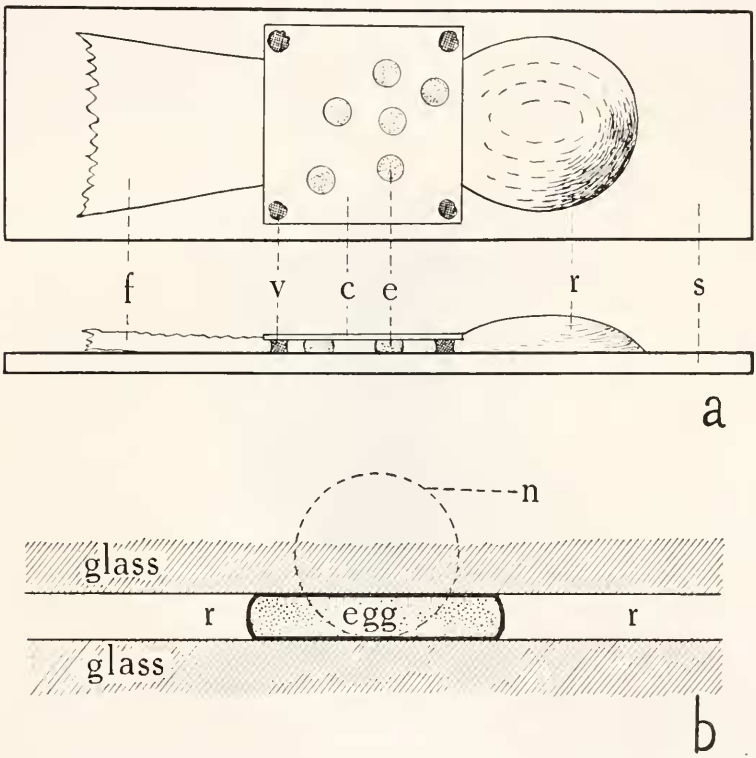

FIGURE 1. a, Diagrammatic view of the arrangement used for the experiments. b, Diagrammatic view of the compressed egg. c, cover-glass; e, egg; f, filter-paper; n, outline of a normal egg; r, reagent to stimulate eggs; s, slide-glass; v, vaseline.

ascertained that the eggs thus deprived of their jelly-coats were fertilizable. A small drop of sea water containing the jelly-less eggs was placed on a slide. A very small amount of vaseline was put on the four corners of a cover-glass and this was placed over the egg-sea water drop. Pushing the cover-glass with a fine needle, the eggs were pressed down and flattened until their diameter reached 160 μ. Since the normal spherical egg has a diameter of about 98 μ, the flattened eggs were considered to take the form schematized in Figure 1b. The upper and lower parts of the eggs adhered closely to the glass-surface, while the equatorial region, so to speak, was in contact with the sea water. It was found possible to control

the amount of vaseline so that there was no movement of the eggs during the subsequent procedure.

Several drops of the reagent to be tested were placed on the slide at one side of the cover-glass (Fig. 1a). A small piece of filter paper was applied to the other side of the cover-glass so that the reagent was drawn into the space beneath the cover-glass. After an appropriate time, the reagent was replaced by normal sea water in a similar way. Thus the equatorial region of the eggs was exposed to the reagent, while the upper and lower regions were not influenced directly by the reagent, since they were closely applied to the glass-surfaces. The cortical granules in the upper part of the eggs were examined with the oil-immersion objective. The appropriate time of exposure of the eggs to each reagent was preliminarily determined by experiments using eggs in watch glasses. The length of time sufficient to induce the breakdown of the cortical granules in the watch glasses was taken as the appropriate exposure time for the experiments.

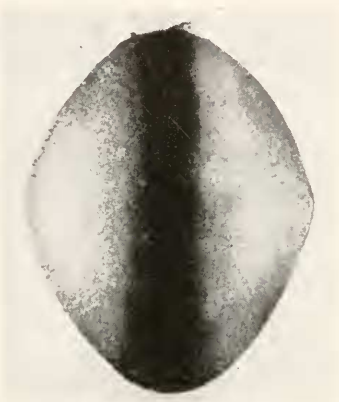

FIGURE 2. An egg, the equator of which was exposed to a solution of Janus green by the method shown in Figure 1.

Experiments were usually repeated more than twenty times for each reagent and the results were found to be quite reproducible. Figure 2 shows an egg, the surface of which was partially exposed to a solution of Janus green by the above-described method. The equatorial region is deeply stained while the other areas remain unstained, indicating that the dye did not soak into these regions. The fact that, as will be shown later, even surface-active substances such as detergents do not exert their influence on the upper part of the eggs indicates that this technique is suitable for the present purpose.

RESULTS

Experiments with wasp-venom, sodium choleinate and detergents

Wasp-venom was obtained from *Polistes fadwigae*. The poison gland was placed in a few drops of sea water and the venom was extracted. Employing the above-described technique, the equatorial regions of the eggs were exposed to the

solution of wasp-venom in sea water. With an appropriate concentration of wasp-venom, the cortical granules in the equatorial region soon began to break down. After they had completely broken down, the upper part of the eggs was examined. It was found that the cortical granules in that part were quite intact and no sign of the influence of the wasp-venom was observed (Fig. 3b). If the solution was replaced by normal sea water after the membrane was elevated on the equatorial region and before cytolysis took place, no cortical change was observed in the upper part of the eggs even after 10 minutes.

Similar experiments were performed using sodium choleinate. To 1 cc. of sea water were added 10 drops of a 1 per cent solution of sodium choleinate. The result with this solution was found to be quite the same as that with wasp-venom.

In the next experiments, the effects of Monogen and Lipon were studied. Monogen is a detergent, consisting chiefly of a mixture of myristyl sulphate and lauryl sulphate. When the eggs were immersed in a 0.5 per cent solution of Monogen in sea water, the breakdown of the cortical granules began in a short

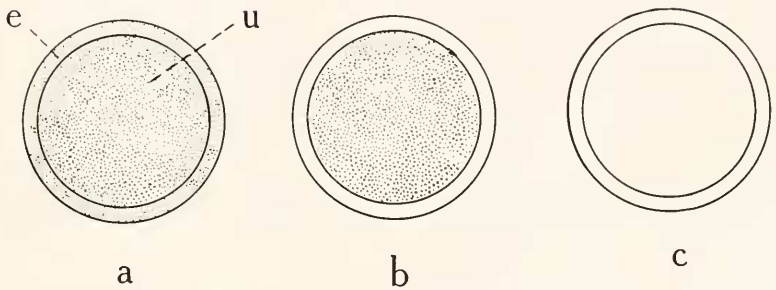

FIGURE 3. Diagrams to show the cortical response of eggs partially exposed to various reagents. a, unexposed egg. The cortical granules are intact all over the egg. b, an egg exposed to wasp-venom, sodium choleinate or Monogen. c, an egg exposed to fatty acid, non-electrolyte or distilled water. e, equatorial region; u, upper part of the egg adhering closely to glass surface.

time and membrane elevation was completed in 2 minutes at 18° C. Too long an exposure to the Monogen solution resulted in cytolysis of the eggs. However, if the eggs were washed with sea water immediately after the membrane was formed, they could be caused to develop into larvae after an appropriate treatment with a hypertonic solution. Thus, Monogen has proved to be an excellent parthenogenetic agent. Lipon, another detergent, consisting chiefly of alkyl sulfonate, has a similar effect. It was found that the result of partial exposure of the egg surface to Monogen or Lipon was almost the same as those with wasp-venom and sodium choleinate.

These results suggest that the cortical change caused by treatment with wasp-venom, sodium choleinate, Monogen and Lipon is not of a propagating nature.

Experiments with fatty acids, non-electrolytes and distilled water

Monobasic fatty acids are known to be very effective in inducing membrane formation in sea urchin eggs. Experiments were performed employing the technique

described above, in order to determine whether the effect of fatty acids is of a propagating nature. The egg surface was partially exposed to butyric acid sea water (47 cc. of sea water plus 3 cc. of N/10 butyric acid) for 30 seconds at 18° C., and then the solution was replaced by normal sea water. After about 20 seconds the cortical granules in the equatorial region of the egg began to break down and a membrane started to separate. The granular breakdown then rapidly proceeded in the upper unexposed part of the egg and within 5 to 9 seconds their breakdown was finished all over the egg surface (Fig. 3c). Acetic and propionic acids also were found to have the same effect. It is apparent that these results are entirely different from those with wasp-venom, sodium choleinate and detergents. It is concluded that the effect of fatty acids is of a propagating nature.

It has been known since the publication of Motomura's work (1934) that urea solution is an excellent activating agent. Moser (1940) has observed that Arbacia eggs treated with molar concentrations of non-electrolyte solutions exhibit the same kind of visible cortical response as that obtained with sperm cells and other stimulating agents. Therefore, it is of interest to determine whether the effect of non-electrolytes is of a propagating nature. Molar solutions (pH 7.0) of urea, sucrose and glycerine were employed. Among these, the urea solution proved the most suitable, since almost 100% of the eggs could be activated. The experimental results were the same as those obtained with fatty acids, indicating that the effect of a non-electrolyte solution is of a propagating nature.

Another series of experiments was undertaken to study the effect of distilled water. It was found that distilled water also was a strong stimulating agent, having the same effect as that of fatty acids.

Discussion

Up to the present, numerous reagents have been found to be effective for inducing the elevation of the fertilization membrane in sea urchin eggs. The range of such effective reagents is exceedingly wide, but in every case the first visible cortical response, regardless of the nature of the reagent, is the breakdown of the cortical granules (Moser, 1939). The elevation of the membrane follows this initial visible cortical response. On the basis of these facts it has been believed by some workers that stimulating reagents other than sperm cells effect essentially the same type of cortical response as that obtained upon insemination. However, the results presented in this paper make it possible to classify the stimulating reagents into two groups according to the nature of their effects. This further means that their effects are not always the same so far as the invisible cortical response is concerned. The first group includes reagents such as butyric acid, distilled water and isotonic solutions of non-electrolytes. The cortical change provoked by these reagents is of a propagating nature. To the second group belong reagents such as wasp-venom, sodium choleinate, Monogen and Lipon. The response to their effects is of a non-propagating nature.

It should be noted here that the propagating nature of the change induced by reagents which belong to the first group is capable of proof only when the egg surface is partially exposed to the reagents. In usual experiments the eggs in any solution are completely exposed to it, so that the propagating nature of the response is not detectable. The question arises, therefore, as to the occurrence of the propa-

gation of the response in such cases. Yamamoto (1944) has shown in Oryzias that there is a gradient of irritability in the cortex of the unfertilized egg. According to him, the irritability is highest at the animal pole, medium at the equator and lowest at the vegetal pole. If it be assumed that there is a gradient of irritability in the cortex of the sea urchin egg also, and in addition that the response occurs first in the most irritable part of the cortex when the egg is immersed in a solution of a stimulating reagent, it would seem probable that the response might travel in a rapid wave-like fashion around the egg cortex before the direct response to the reagent occurred in the other part. At present, however, no evidence has been furnished as to such a gradient of irritability in the sea urchin egg. Therefore, there remains another possibility, that the cortical response in all parts of the egg surface takes place at the same time, being directly provoked by the chemical stimulus. However, regardless of this, the response induced by a reagent of the first group should be considered to be essentially different from that induced by reagents of the second group, since the former was able to propagate over a part of the egg surface not exposed to the reagent, while the latter was not.

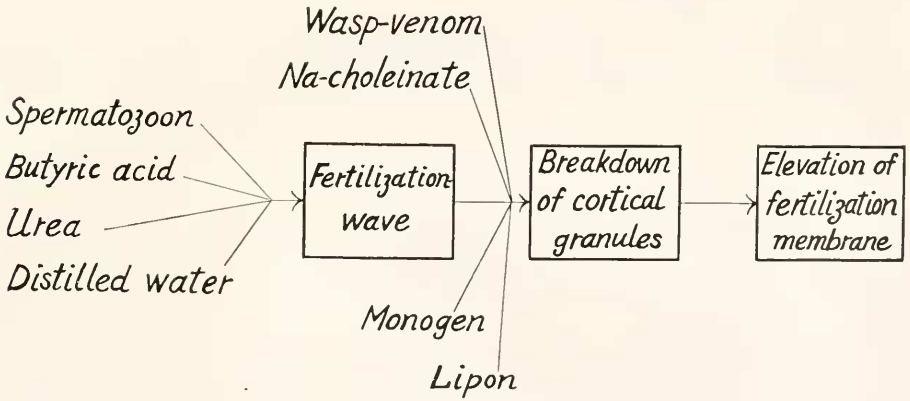

FIGURE 4. Diagram showing the process of the cortical change.

In the first paper of this series it was shown that the breakdown of one cortical granule does not automatically induce the breakdown of its neighbours. The author is therefore convinced that there must be an invisible wave-like change underlying the breakdown of the cortical granules whenever the cortical change is of a propagating nature. According to this concept, reagents such as urea or butyric acid stimulate the eggs to initiate an invisible wave-like change, which is followed by the breakdown of the cortical granules.

In the previous paper it was also postulated that some invisible cortical change of a propagating nature must occur at the time of fertilization. This invisible cortical change was called the "fertilization-wave." The nature of the cortical change provoked by the reagents of the first group discussed in this paper suggests that this change is essentially the same as that resulting from the entrance of spermatozoa. If this suggestion be true, then it may be said that the reagents of the first group induce the fertilization-wave itself, while the reagents of the second group in-

duce the same changes as the fertilization-wave, without the intervention of the fertilization-wave. This conclusion is essentially in agreement with the view considered on the effect of wasp-venom and sodium choleinate in the previous paper.

These concepts give us the scheme diagrammed in Figure 4. It shows that the spermatozoon and the reagents of the first group give rise to the fertilization-wave and this causes the breakdown of the cortical granules. The reagents of the second group cause the breakdown of the cortical granules without the intervention of the fertilization-wave. The granular breakdown, then, is followed by the elevation of the fertilization membrane, regardless of the nature of the activating reagent.

In a recent paper, Runnström and Kriszat (1952) reported that in Psammechinus eggs the propagation of the impulse caused by the attachment of the spermatozoon is inhibited by the attachment of the egg surface to glass. The data on Hemicentrotus eggs do not agree with these findings. Either there is a great difference in these two sea urchins with respect to the nature of the cortex, or the dissimilarities must be accounted for by differences in the conditions under which the observations were made.

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Summary

1. A special technique has been developed for partial exposure of a sea urchin egg surface to stimulating reagents under microscopical observation.

2. The surfaces of Hemicentrotus eggs were partially exposed to stimulating reagents for an appropriate time and the unexposed surface was examined after washing with sea water.

3. When wasp-venom, sodium choleinate, Monogen or Lipon was used, the cortical granules in the exposed cortex were completely broken down within a few minutes, while those in the unexposed cortex remained quite intact, showing no sign of the influence of the reagent. The effect of these reagents is believed to be of a non-propagating nature.

4. When butyric acid, acetic acid, propionic acid, distilled water or an isotonic solution of a non-electrolyte was used, the granular breakdown proceeded rapidly in the unexposed part of the cortex immediately after granule breakdown in the exposed part. Therefore, it is concluded that these reagents induce a cortical change of a propagating nature.

5. It is suggested that the nature of the cortical change provoked by reagents of the latter group is essentially the same as that which follows the entrance of spermatozoa.

LITERATURE CITED

Moser, F., 1939. Studies on a cortical layer response to stimulating agents in the Arbacia egg. II. Response to chemical and physical agents. *J. Exp. Zool.*, **80**: 447–471.

Moser, F., 1940. Studies on a cortical layer response to stimulating agents in the Arbacia egg. III. Response to non electrolytes. *Biol. Bull.*, **78**: 68–79.

MOTOMURA, I., 1934. On the mechanism of fertilization and development without membrane formation in the sea urchin egg, with notes on a new method of artificial parthenogenesis. *Sci. Rep. Tohoku Imp. Univ., B,* **9**: 33–45.

RUNNSTRÖM, J., AND G. KRISZAT, 1952. The cortical propagation of the activation impulse in the sea urchin egg. *Exp. Cell Res.,* **3**: 419–426.

SUGIYAMA, M., 1952. Physiological analysis of the cortical response of the sea urchin egg to stimulating reagents. I. Response to sodium choleinate and wasp-venom. *Biol. Bull.,* **104**: 210–215.

YAMAMOTO, T., 1944. Physiological studies on fertilization and activation of fish eggs. II. The conduction of the "fertilization-wave" in the egg of *Oryzias latipes*. *Ann. Zool. Jap.,* **22**: 109–136.